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(54) Title: NUCLEIC ACIDS ENCODING INTERFERON GAMMA INDUCING FACTOR-2		
(57) Abstract		
<p>The present invention provides a polynucleotide (igif-2) which identifies and encodes a novel interferon gamma inducing factor-2 (IGIF-2) which was expressed in adenoid, brain, kidney, liver, lung, skin, synovium, and T-lymphocytes. The present invention also provides for antisense molecules. The invention further provides genetically engineered expression vectors and host cells for the production of purified IGIF-2; antibodies, antagonists and inhibitors; and pharmaceutical compositions and methods of treatment based on the polypeptide, its antibodies, antagonists and inhibitors. The invention specifically provides for use of the polypeptide as therapeutic for immuno-compromised individuals and as a positive control in diagnostic assays for the detection of aberrant IGIF-2 expression or altered leukocyte or lymphocyte activity.</p>		
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NUCLEIC ACIDS ENCODING INTERFERON GAMMA INDUCING FACTOR-2

TECHNICAL FIELD

The present invention relates to nucleic acid and amino acid sequences of a novel cytokine, interferon gamma inducing factor-2 and to the use of these sequences in the diagnosis, study, prevention and treatment of disease.

BACKGROUND ART

Cytokines are active in cell proliferation, differentiation and movement at picomolar to nanomolar concentrations and have effects on such activities as leukocyte migration and function, hematopoietic cell numbers, temperature regulation, acute response to infections, tissue remodeling and cell survival. Since cytokines are produced in groups and in patterns characteristic of the particular stimulus or disease, studies using antibodies or other drugs that modify the activity of a particular cytokine are beginning to elucidate the roles of individual cytokines in pathology and physiology. For purposes of example, two cytokines that are rapidly expressed in response to inflammation, interferon gamma (IFN- γ) and interleukin-12 (IL-12), will be described.

IFN- γ is a pleiotropic cytokine involved in the regulation of immune and inflammatory responses. It is produced by CD4⁺, CD8⁺, and Th1 lymphocytes and natural killer cells in response to antigens or mitogens. IFN- γ is a homodimer formed by the antiparallel association of two subunits, each with six α helices held together by short non-helical sequences. The overall structure of this 40-70 kD dimer is globular, and it contains two potential N-glycosylation sites. The single gene which encodes IFN- γ is located on the long arm of chromosome 12, and its low homology with sequences from other species accounts for species specific activity. IFN- γ participates in the activation, growth and differentiation of T, B, natural killer and endothelial cells, macrophages and fibroblasts. IFN- γ enables IgG2 α production, potentiates the antiviral and antiproliferative activity of IFN α/β , increases expression of class II major histocompatibility complex molecules on B cells and macrophages, and inhibits IgG1 and IgE production, Th2 cell proliferation, and the effects of IL-3, IL-4, granulocyte-monocyte colony stimulating factor (GM-CSF) and TNF- α on bone marrow cells. In

model studies of septic shock and cerebral malaria. IFN- γ has exacerbated disease pathology by generating toxic levels of TNF- α .

IL-12 is composed of two disulfide linked chains of 35 and 40 kD which form a biologically active heterodimer. Neither chain is closely related to known proteins. The 40 kD chain, which shows 70% homology between man and mouse, appears to be species specific and contains sequence motifs present in the Ig superfamily and the hematopoietin family of receptors. The known functions of IL-12 include activation of cytotoxic lymphocytes, induction of IFN- γ synthesis by T cells and natural killer cells, mediation of the Th-1 response to antigenic challenge, growth factor-like stimulation of activated CD4⁺T cells, CD8⁺T cells, and natural killer cells, and acceleration of the lytic activity of natural killer and lymphocyte or lymphokine activated killer cells.

IFN- γ inducing factor (IGIF) is a recently discovered cytokine from mouse liver (Okamura H et al. (1995) Nature 378:88-91). The IGIF gene encodes a unique precursor protein of 192 amino acids and a mature protein of 157 amino acids which has no glycosylation sites. The mRNA for IGIF has been found in Kupffer cells and activated macrophages, and the recombinant protein is a stronger inducer of IFN- γ than IL-12. Investigations with IL-12 and IGIF suggest that these proteins may work synergistically to induce Th-1 production of IFN- γ ; however, antibody studies demonstrate that IGIF and IL-12 use different pathways to carry out this induction. Additionally, Okamura ^{supra} demonstrated that administration of anti-IGIF antibody prevented liver damage in mice treated with bacteria and challenged with lipopolysaccharides to induce toxic shock. Okamura et al. suggest that IGIF may decrease leukocyte initiated tissue destruction in the liver and that modulation of IGIF may have other applications in inflammatory diseases such as AIDS, pulmonary tuberculosis, and leprosy.

Current techniques for the diagnosis of abnormalities in inflamed or diseased tissues mainly rely on clinical observations or serological analyses of body fluids or tissues for hormones, polypeptides or various metabolites. Mammals, however, may not manifest clinical symptoms early in the inflammation and disease process, and serological analyses do not always differentiate between invasive diseases and genetic syndromes which have similar or overlapping ranges. Thus, development of new diagnostic methods for the detection of inflammation and disease states would be important in providing early

and accurate diagnosis, understanding molecular pathogenesis, and developing effective therapies.

Cytokines are reviewed, inter alia, in Callard RE and AJH Gearing (1994) The Cytokine Factsbook, Academic Press, New York NY, Guyton, AC (1991) Textbook of Medical Physiology, WB Saunders Co, Philadelphia PA, and Paul WE (1993) Fundamental Immunology, Raven Press, New York NY.

DISCLOSURE OF THE INVENTION

The present invention relates to a novel cytokine, interferon gamma inducing factor-2 (IGIF-2), found in cDNA libraries made from human adenoid, brain, kidney, liver, lung, skin, synovium, and T-lymphocytes, and to the use of the nucleic acid and amino acid sequences of this novel cytokine in the study, diagnosis, prevention and treatment of inflammation and disease.

IGIF-2 disclosed herein was first identified in Incyte Clone 631796 through a computer generated search for amino acid sequence alignments. The present invention has a length of 193 amino acids and approximately 60% amino acid similarity to the Mus musculus amino acid sequence of interferon gamma inducing factor (IGIF; Okamura H et al. (1995) Nature 378:88-91; GI 1064823). The nucleic acid sequence (shown in lower case, igif-2), SEQ ID NO: 1, and amino acid sequence (shown in upper case, IGIF-2), SEQ ID NO: 2, are disclosed herein.

Also disclosed herein is an IGIF-2 variant containing a point mutation at nucleotide 622 of SEQ ID NO:1 resulting in an amino acid substitution at residue 140. Igif-2 from the T-lymphocyte cDNA library has a guanine (G) at nucleotide position 622 of SEQ ID NO:1 which encodes an arginine while Igif-2 from the liver cDNA library has a thymine (T) at nucleotide position 622 which encodes an isoleucine at amino acid residue 140. Also disclosed herein is an IGIF-2 variant found in inflamed adenoid which is 42 amino acids in length and shares the N-terminal 30 amino acid residues of IGIF-2 (SEQ ID NO:2), the additional amino acid residues (in single letter code) being GKVEMNLFFFAN (SEQ ID NO:3). This Igif-2 variant has a poly. A tail and may represent an alternatively spliced Igif-2 transcript.

IGIF has been shown to induce IFN- γ , a cytokine involved in regulating the

immune response and shown to exacerbate disease pathology by generating toxic levels of TNF- α . Administration of anti-IGIF has been shown to prevent liver damage in mice treated with bacteria and challenged with lipopolysaccharides to induce toxic shock. Therefore, nucleic acid and amino acid sequences of the present invention will provide the basis for the development of diagnostic and treatment methods for the early and accurate detection and treatment of disease states and conditions associated with inflammation and/or the expression of IGIF-2. Furthermore, the nucleic acid and amino acid sequences disclosed herein will provide the basis for diagnostic and therapeutic compositions for the detection and treatment of disease states and conditions associated with inflammation and/or the expression of IGIF-2.

The polynucleotide sequence disclosed herein which encodes IGIF-2, or variants thereof, provides the basis for designing oligonucleotide probes for the diagnosis of disease and conditions associated with inflammation and/or the expression of IGIF-2. Such probes may be used to diagnose inflammation and tissue destruction in cells and tissues before the onset of severe clinical symptoms. The invention also provides for igif-2 antisense molecules which may be used to diminish or eliminate expression of genomic igif-2 nucleotide sequences in individuals subject to an overactive or inappropriate immune response such as in allergies and asthma. The present invention also relates, in part, to expression vectors and host cells comprising igif-2 for in vitro or in vivo production of IGIF-2.

The present invention also relates to the use of IGIF-2, or fragments or variants thereof, to produce anti-IGIF-2 antibodies and to screen for antagonists or inhibitors of IGIF-2 which can be used therapeutically to prevent IGIF-2 induction of proliferation, differentiation, and maturation of leukocytes and lymphocytes. Such antagonists or inhibitors can be used to downregulate the immune response thereby preventing the secretion of proteolytic enzymes which may cause profound tissue damage.

The present invention further relates to administration of compositions comprising purified IGIF-2 or variants thereof, to immune-compromised individuals, such as individuals subject to HIV, for the purpose of inducing endogenous antiviral molecules, such as IFN- γ , and for inducing proliferation, differentiation and maturation of leukocytes and lymphocytes.

The present invention also relates to compositions comprising anti-IGIF-2 antibodies, or other antagonists or inhibitors for the diagnosis, prevention or treatment of inherited or acquired diseases, involving the abnormal expression of igif-2 or altered leukocyte or lymphocyte activity. Such conditions would include such as viral (AIDS, hepatitis), bacterial (septic shock), fungal (histoplasmosis) or helminthic infections; allergies or asthma; mechanical injury through exposure (to asbestos, coal dust, etc) or trauma; arteriosclerosis, atherogenesis or collagen vascular diseases; hereditary diseases such as autoimmune hemolytic anemia, biliary cirrhosis, juvenile diabetes mellitus, lupus erythematosus, multiple sclerosis, myasthenia gravis, or rheumatoid arthritis; leukemia, lymphomas or carcinomas; Crohn's or other inflammatory bowel diseases; or other conditions which involve the abnormal activity of leukocytes or lymphocytes.

The igif-2 polynucleotide sequences disclosed herein, oligonucleotides, fragments, portions or antisense molecules thereof, may be used in diagnostic assays to detect and quantify levels of igif-2 mRNA in cells and tissues. For example, the igif-2 polynucleotide sequence may be used to detect related or identical sequences in solution-based, membrane-based, or tissue-based assays to diagnose abnormalities in gene expression. The invention further provides diagnostic assays and kits for the detection of IGIF-2 in cells and tissues comprising purified IGIF-2, which may be used as a positive control, and anti-IGIF-2 antibodies. Such antibodies may be used in solution-based, membrane-based, or tissue-based technologies to detect any disease state or condition related to the abnormal expression of IGIF-2 or altered leukocyte or lymphocyte activity.

Igif-2 antisense molecules, anti-IGIF-2 antibodies, antagonists or inhibitors of IGIF-2 may be used for therapeutic purposes, for example, in inhibiting or neutralizing overexpression of IGIF-2 associated with inflammation, for example, in individuals subject to hepatitis or pancreatitis. The present invention provides pharmaceutical compositions for the treatment of disease states associated with abnormal expression of igif-2 or altered leukocyte or lymphocyte activity. Such pharmaceutical compositions will comprise effective amounts of antisense molecules capable of inhibiting transcription and/or translation of genomic polynucleotide sequences, anti-IGIF-2 antibodies, or antagonists or inhibitors of IGIF-2. Alternatively, the present invention also provides pharmaceutical compositions comprising effective amounts of IGIF-2 polypeptide, or

variants thereof. for the treatment of immune-compromised individuals. such as individuals subject to HIV, for the purpose of inducing endogenous antiviral molecules, such as IFN- γ , and for inducing proliferation, differentiation and maturation of leukocytes and lymphocytes.

5 The present invention also encompasses the use of gene therapy methods for the introduction of nucleotide sequences of the present invention into individuals subject to diseases or conditions associated with immune response.

BRIEF DESCRIPTION OF DRAWINGS

10 Figures 1A and B displays the nucleic acid and amino acid sequences of interferon gamma inducing factor-2. The alignment was produced using MacDNASIS PRO™ software (Hitachi Software Engineering Co., Ltd., San Bruno, CA).

15 Figure 2 shows the amino acid sequence similarity between IGIF-2 (631796; SEQ ID NO:2) and IGIF (GI 1064823; SEQ ID NO:4). Sequences shown were produced using the multisequence alignment program of DNASTAR™ software (DNASTAR Inc, Madison WI)

20 Figure 3 shows an image of the laboratory northern analysis of IGIF-2. The left side of the image shows standard size markers, and the bottom shows the numbered lanes which contain nucleic acids extracted from: 1) spleen, 2) lymph node, 3) thymus, 4) appendix, 5) peripheral blood, 6) bone marrow, and 7) fetal liver. The IGIF-2 band appears in various lanes across the image at approximately the same level as the 1.35 standard molecular weight marker.

25 Figure 4 shows the electronic northern analysis of IGIF-2 produced using Incyte clone 631796 and the LIFESEQ™ database (Incyte Pharmaceuticals Inc., Palo Alto, CA).

MODES FOR CARRYING OUT THE INVENTION

30 The present invention relates to a novel cytokine referred to herein as "interferon gamma inducing factor-2 " which was found expressed in cDNA libraries made from human kidney, liver, T lymphocytes and inflamed adenoid. As used herein, the abbreviation for the novel interferon gamma inducing factor-2 in lower case (igif-2) refers to a nucleic acid sequence while the upper case (IGIF-2) refers to an amino acid sequence.

"Nucleic acid sequence" as used herein refers to an oligonucleotide, nucleotide or

polynucleotide sequence, and fragments or portions thereof, and to DNA or RNA of genomic or synthetic origin which may be double-stranded or single-stranded whether representing the sense or antisense strand. Similarly, "amino acid sequence" as used herein refers to peptide or protein sequences or portions thereof.

5 As used herein, IGIF-2 refers to IGIF-2 from any species, including, bovine, ovine, porcine, equine, and preferably human, in naturally occurring or in variant form, or from any source, whether natural, synthetic, semi-synthetic or recombinant. A preferred IGIF-2 variant is one having at least 80% amino acid sequence similarity, another preferred IGIF-2 variant is one having at least 90% amino acid sequence similarity and
10 another preferred IGIF-2 variant is one having at least 95% amino acid sequence similarity to the IGIF-2 amino acid sequence illustrated in Figure 1 (SEQ ID NO:2). A preferred IGIF-2 variant of the present invention is one having isoleucine at amino acid position 140 of SEQ ID NO:2. Another preferred IGIF-2 variant is one having the first 30 amino acid residues of SEQ ID NO:2 with additional amino acids GKVEMNLFFFAN
15 (SEQ ID NO:3) terminating at amino acid residue 42.

 IGIF-2 is a cytokine which is 60% homologous to IGIF at the amino acid level (GI 1064823; Okamura H et al. (1995) Nature 378:88-91) as shown in the consensus sequences in Figure 2. Cytokines are involved in leukocyte and lymphocyte cell proliferation, differentiation, and movement, have effects on hematopoietic cell numbers,
20 temperature regulation, acute response to infections, tissue remodeling and cell survival, and are known to be produced by damaged or stressed cells as well as cells of the immune system.

 As used herein, "naturally occurring" refers to an IGIF-2 with an mRNA sequence found in nature, and "biologically active" refers to an IGIF-2 having structural, regulatory
25 or biochemical functions of the naturally occurring IGIF-2. Likewise, "immunological activity" is defined as the capability of the natural, recombinant or synthetic IGIF-2, or any oligopeptide thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies.

 The term "derivative" as used herein refers to the chemical modification of an igif-
30 2 or the encoded IGIF-2. Illustrative of such modifications would be replacement of hydrogen by an alkyl, acyl, or amino group. An igif-2 derivative would encode a

polypeptide which retains essential biological characteristics of IGIF-2 such as. for example, the differentiation of monocytes.

As used herein, the term "purified" refers to molecules, either nucleic or amino acid sequences, that are removed from their natural environment and isolated or separated from at least one other component with which they are naturally associated.

The IGIF-2 Coding Sequences

The nucleic acid (SEQ ID NO:1) and deduced amino acid sequences (SEQ ID NO:2) of IGIF-2 are shown in Figure 1. In accordance with the invention, any nucleotide sequence which encodes the amino acid sequence of IGIF-2 can be used to generate recombinant molecules which express IGIF-2. Nucleotide sequences for igif-2 were identified through BLAST analysis of the N-terminal amino acid sequence deduced from Incyte Clone 631796 derived from the kidney cDNA library. The nucleic acid sequence of SEQ ID NO:1 was assembled using Incyte Clones: 631796 from the kidney cDNA library (designated KIDNNOT05); 450202 from the T- lymphocyte cDNA library (designated TLYMNOT02); and 89908 from the liver cDNA library (designated LIVRNOT01). Incyte clone 159939 from the inflamed adenoid library (designated ADENINB01) is an exact match to Incyte Clone 631796 from nucleotides 30 to 294 and has a poly A+ tail. This clone appears to represent an alternatively spliced IGIF-2 transcript. In IGIF-2 from the T-lymphocyte cDNA library, the nucleotide at position 622 of the nucleic acid sequence is G which would code for the arginine shown at position 140 in the amino acid sequence; however, at the same position in IGIF-2 from the liver cDNA library, the nucleotide at position 622 is T, and the amino acid would be isoleucine. Neither of these changes produce the lysine found at nucleotide position 622 in mouse and may represent a polymorphic codon.

Methods for DNA sequencing are well known in the art and employ such enzymes as the Klenow fragment of DNA polymerase I, Sequenase® (US Biochemical Corp, Cleveland OH)), Taq polymerase (Perkin Elmer, Foster City CA), thermostable T7 polymerase (Amersham, Chicago IL), or combinations of recombinant polymerases and proofreading exonucleases such as the ELONGASE™ Amplification system marketed by Gibco BRL (Gaithersburg MD) Methods to extend the DNA from an oligonucleotide

primer annealed to the DNA template of interest have been developed for both single-stranded and double-stranded templates. Chain termination reaction products were separated using electrophoresis and detected via their incorporated, labeled precursors. Recent improvements in mechanized reaction preparation, sequencing and analysis have permitted expansion in the number of sequences that can be determined per day. Preferably, the process is automated with machines such as the Hamilton Micro Lab 2200 (Hamilton, Reno NV), Peltier Thermal Cycler (PTC200; MJ Research, Watertown MA) and the Applied Biosystems (Foster City CA) Catalyst 800 and 377 and 373 DNA sequencers.

The quality of any particular cDNA library may be determined by performing a pilot scale analysis of the cDNAs and checking for percentages of clones containing vector, lambda or *E. coli* DNA, mitochondrial or repetitive DNA, and clones with exact or homologous matches to public databases.

Extending igif-2 Polynucleotide Sequence

The polynucleotide sequence of igif-2 may be extended utilizing partial nucleotide sequences from SEQ ID NO:1 and various methods known in the art to procure upstream sequences such as promoters and regulatory elements. Sarkar, G. (1993; PCR Methods Applic. 2:318-322) discloses "restriction-site polymerase chain reaction (PCR)" as a direct method which uses universal primers to retrieve unknown sequence adjacent to a known locus. First, genomic DNA is amplified in the presence of primer to a linker sequence and a primer specific to the known region. The amplified sequences are subjected to a second round of PCR with the same linker primer and another specific primer internal to the first one. Products of each round of PCR are transcribed with an appropriate RNA polymerase and sequenced using reverse transcriptase.

Inverse PCR can be used to amplify or extend sequences using divergent primers based on a known region (Triglia T et al(1988) Nucleic Acids Res 16:8186). The primers may be designed using Oligo 4.0 (National Biosciences Inc, Plymouth MN), or another appropriate program, to be 22-30 nucleotides in length, to have a GC content of 50% or more, and to anneal to the target sequence at temperatures about 68°-72° C. The method uses several restriction enzymes to generate a suitable fragment in the known region of a

gene. The fragment is then circularized by intramolecular ligation and used as a PCR template.

Capture PCR (Lagerstrom M et al (1991) PCR Methods Applic 1:111-19) is a method for PCR amplification of DNA fragments adjacent to a known sequence in human and yeast artificial chromosome (YAC) DNA. Capture PCR also requires multiple restriction enzyme digestions and ligations to place an engineered double-stranded sequence into an unknown portion of the DNA molecule before PCR.

Parker JD et al (1991; Nucleic Acids Res 19:3055-60), teach walking PCR, a method for targeted gene walking which permits retrieval of unknown sequence. The PromoterFinder™ kit available from Clontech (Palo Alto CA) uses PCR, nested primers and PromoterFinder libraries to walk in genomic DNA. This process avoids the need to screen libraries and is useful in finding intron/exon junctions.

Another PCR method, "Improved Method for Obtaining Full Length cDNA Sequences" by Guegler et al, Patent Application Serial No 08/487,112, filed June 7, 1995 and hereby incorporated by reference, employs XL-PCR™ (Perkin-Elmer, Foster City CA) to amplify and/or extend nucleotide sequences.

Preferred libraries for screening for full length cDNAs are ones that have been size-selected to include larger cDNAs. Also, random primed libraries are preferred in that they will contain more sequences which contain the 5' and upstream regions of genes. A randomly primed library may be particularly useful if an oligo d(T) library does not yield a full-length cDNA. Genomic libraries are useful for obtaining introns and extending 5' sequence.

A new method for analyzing either the size or confirming the nucleotide sequence of sequencing or PCR products is capillary electrophoresis. Systems for rapid sequencing are available from Perkin Elmer, Beckman Instruments (Fullerton CA), and other companies. Capillary sequencing employs flowable polymers for electrophoretic separation, four different fluorescent dyes (one for each nucleotide) which are laser activated, and detection of the emitted wavelengths by a charge coupled device camera. Output/light intensity is converted to electrical signal using appropriate software (eg. Genotyper™ and Sequence Navigator™ from Perkin Elmer) and the entire process from loading of samples to computer analysis and electronic data display is computer

controlled. Capillary electrophoresis is particularly suited to the sequencing of small pieces of DNA which might be present in limited amounts in a particular sample. The reproducible sequencing of up to 350 bp of M13 phage DNA in 30 min has been reported (Ruiz-Martinez MC et al (1993) Anal Chem 65:2851-8).

5

Expression of igif-2

In accordance with the present invention, igif-2 polynucleotide sequences which encode IGIF-2, fragments, fusion proteins or functional equivalents thereof, may be used to generate recombinant DNA molecules that direct the expression of IGIF-2 in appropriate host cells. Due to the inherent degeneracy of the genetic code, DNA sequences other than the nucleotide sequences of SEQ ID NO:1 which encode substantially the same or a functionally equivalent amino acid sequence, may be used to clone and express IGIF-2. As will be understood by those of skill in the art, it may be advantageous to produce IGIF-2-encoding nucleotide sequences possessing non-naturally occurring codons. Codons preferred by a particular prokaryotic or eukaryotic host (Murray E et al (1989) Nuc Acids Res 17:) can be selected, for example, to increase the rate of IGIF-2 expression or to produce recombinant RNA transcripts having desirable properties, such as a longer half-life, than transcripts produced from naturally occurring sequence.

Also included within the scope of the present invention are polynucleotide sequences that are capable of hybridizing to the nucleotide sequence of Figure 1 under conditions of intermediate to maximal stringency. Hybridization conditions are based on the melting temperature (T_m) of the nucleic acid binding complex, as taught in Wahl GM et al. (1987, Methods Enzymol 152:399-407) incorporated herein by reference, and confer a defined "stringency" as explained below.

"Maximum stringency" typically occurs at about $T_m - 5^\circ\text{C}$ (5°C below the T_m of the probe); "high stringency" at about 5°C to 10°C below T_m ; "intermediate stringency" at about 10°C to 20°C below T_m ; and "low stringency" at about 20°C to 25°C below T_m . As will be understood by those of skill in the art, a maximum stringency hybridization can be used to identify or detect identical polynucleotide sequences while an intermediate (or low) stringency hybridization can be used to identify or detect similar

or related polynucleotide sequences.

The term "hybridization" as used herein refers to "the process by which a strand of nucleic acid joins with a complementary strand through base pairing" (Coombs J (1994) Dictionary of Biotechnology, Stockton Press, New York NY). Amplification as carried out in polymerase chain reaction technologies is described in Dieffenbach CW and GS Dveksler (1995, PCR Primer, a Laboratory Manual, Cold Spring Harbor Press, Plainview NY) and incorporated herein by reference.

As used herein a "deletion" is defined as a change in either nucleotide or amino acid sequence in which one or more nucleotides or amino acid residues, respectively, are absent.

As used herein an "insertion" or "addition" is that change in a nucleotide or amino acid sequence which has resulted in the addition of one or more nucleotides or amino acid residues, respectively, as compared to the naturally occurring molecules.

As used herein "substitution" results from the replacement of one or more nucleotides or amino acids by different nucleotides or amino acids, respectively.

Variant igif-2 polynucleotide sequences may be used in accordance with the invention and include deletions, insertions or substitutions of different nucleotide residues resulting in a polynucleotide that encodes the same or a functionally equivalent IGIF-2. Variant IGIF-2 protein may also be used in accordance with the invention and may include deletions, insertions or substitutions of amino acid residues as long as the result is a functionally equivalent IGIF-2.

Amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues as long as the biological activity of IGIF-2 is retained. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include leucine, isoleucine, valine; glycine, alanine; asparagine, glutamine; serine, threonine, phenylalanine, and tyrosine.

Included within the scope of the present invention are alleles of igif-2. As used herein, an "allele" or "allelic sequence" is an alternative form of igif-2. Alleles result from a mutation, i.e., a change in the nucleic acid sequence, and generally produce altered

mRNAs or polypeptides whose structure or function may or may not be altered. Any given gene may have none, one or many allelic forms. Common mutational changes which give rise to alleles are generally ascribed to deletions, additions or substitutions of nucleic acids. Each of these types of changes may occur alone, or in combination with the others, one or more times in a given sequence.

The nucleotide sequences of the present invention may be engineered in order to alter an igif-2 coding sequence for a variety of reasons, including but not limited to, alterations which modify the cloning, processing and/or expression of the gene product. For example, mutations may be introduced using techniques which are well known in the art, eg, site-directed mutagenesis to insert new restriction sites, to alter glycosylation patterns, to change codon preference, etc.

In another embodiment of the invention, an igif-2 natural, modified or recombinant sequence may be ligated to a heterologous sequence to encode a fusion protein. For example, for screening of peptide libraries for inhibitors of IGIF-2 activity, it may be useful to encode a chimeric IGIF-2 protein expressing a heterologous epitope that is recognized by a commercially available antibody. A fusion protein may also be engineered to contain a cleavage site located between an IGIF-2 sequence and the heterologous protein sequence, so that the IGIF-2 may be cleaved and purified away from the heterologous moiety.

In an alternate embodiment of the invention, the coding sequence of igif-2 could be synthesized, whole or in part, using chemical methods well known in the art (See Caruthers et al (1980) Nuc Acids Res Symp Ser 7:215-233; Crea and Horn (1980) Nuc Acids Res 9:2331; Matteucci and Caruthers (1980) Tetrahedron Lett 21:719; and Chow and Kempe (1981) Nuc Acids Res 9:2807-2817). Alternatively, the protein itself could be produced using chemical methods to synthesize an IGIF-2 amino acid sequence, whole or in part. For example, peptides can be synthesized by solid phase techniques, cleaved from the resin, and purified by preparative high performance liquid chromatography (eg, Creighton T (1983) Proteins Structures And Molecular Principles, WH Freeman and Co, New York NY). The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing (eg, the Edman degradation procedure: Creighton, supra)

Direct peptide synthesis can be performed using various solid-phase techniques

(Roberge JY et al (1995) Science 269:202-204) and automated synthesis may be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer in accordance with the instructions provided by the manufacturer. Additionally the amino acid sequence of IGIF-2, or any part thereof, may be altered during direct synthesis and/or
5 combined using chemical methods with other cytokine sequences, or any part thereof, to produce a variant polypeptide.

Expression Systems

In order to express a biologically active IGIF-2, the nucleotide sequence coding for IGIF-2, or a functional equivalent, is inserted into an appropriate expression vector, ie,
10 a vector which contains the necessary elements for the transcription and translation of the inserted coding sequence.

Methods which are well known to those skilled in the art can be used to construct expression vectors containing an igif-2 coding sequence and appropriate transcriptional or
15 translational controls. These methods include in vitro recombinant DNA techniques, synthetic techniques and in vivo recombination or genetic recombination. Such techniques are described in Sambrook, J. et al. (1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Plainview, NY, and Ausubel, F.M. et al. (1989) Current Protocols in Molecular Biology, John Wiley & Sons, New York, NY.

A variety of expression vector/host systems may be utilized to contain and express an igif-2 coding sequence. These include but are not limited to bacteria transformed with recombinant bacteriophage, plasmid or cosmid DNA expression vectors; yeast
20 transformed with yeast expression vectors; insect cell systems infected with virus expression vectors (eg, baculovirus); plant cell systems transfected with virus expression
25 vectors (eg, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with bacterial expression vectors (eg, Ti or pBR322 plasmid); or animal cell systems.

The "control elements" or "regulatory sequences" of these systems vary in their strength and specificities and are those nontranslated regions of the vector, enhancers,
30 promoters, and 3' untranslated regions, which interact with host cellular proteins to carry out transcription and translation. Depending on the vector system and host utilized, any

number of suitable transcription and translation elements, including constitutive and inducible promoters, may be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the Bluescript® phagemid (Stratagene, LaJolla CA) and ptrp-lac hybrids and the like may be used. The baculovirus polyhedrin promoter may be used in insect cells. Promoters or enhancers derived from the genomes of plant cells (eg, heat shock, RUBISCO; and storage protein genes) or from plant viruses (eg, viral promoters or leader sequences) may be cloned into the vector. In mammalian cell systems, promoters from the mammalian genes or from mammalian viruses are most appropriate. If it is necessary to generate a cell line that contains multiple copies of igif-2, vectors based on SV40 or EBV may be used with an appropriate selectable marker.

In bacterial systems, a number of expression vectors may be selected depending upon the use intended for IGIF-2. For example, when large quantities of IGIF-2 are needed for the induction of antibodies, vectors which direct high level expression of fusion proteins that are readily purified may be desirable. Such vectors include, but are not limited to, the *E. coli* cloning and expression vector Bluescript® (Stratagene), in which the igif-2 coding sequence may be ligated into the vector in frame with sequences for the amino-terminal Met and the subsequent 7 residues of β -galactosidase so that a hybrid protein is produced; pIN vectors (Van Heeke & Schuster (1989) J Biol Chem 264:5503-5509); and the like. pGEX vectors (Promega, Madison WI) may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Proteins made in such systems are designed to include heparin, thrombin or factor XA protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at will.

In the yeast, Saccharomyces cerevisiae, a number of vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase and PGH may be used. For reviews, see Ausubel et al (supra) and Grant et al (1987) Methods Enzymol 153:516-544.

In cases where plant expression vectors are used, the expression of an IGIF-2

coding sequence may be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of CaMV (Brisson et al (1984) Nature 310:511-514) may be used alone or in combination with the omega leader sequence from TMV (Takamatsu et al (1987) EMBO J 6:307-311). Alternatively, plant promoters such as the small subunit of RUBISCO (Coruzzi et al (1984) EMBO J 3:1671-1680; Broglie et al (1984) Science 224:838-843); or heat shock promoters (Winter J and Sinibaldi RM (1991) Results Probl Cell Differ 17:85-105) may be used. These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. For reviews of such techniques, see Hobbs S or Murry LE in McGraw Yearbook of Science and Technology (1992) McGraw Hill New York NY, pp 191-196 or Weissbach and Weissbach (1988) Methods for Plant Molecular Biology, Academic Press, New York NY, pp 421-463.

An alternative expression system which could be used to express igif-2 is an insect system. In one such system, Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in Spodoptera frugiperda cells or in Trichoplusia larvae. The igif-2 coding sequence may be cloned into a non-essential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of igif-2 will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein. The recombinant viruses are then used to infect S. frugiperda cells or Trichoplusia larvae in which IGIF-2 is expressed (Smith et al (1983) J Virol 46:584; Engelhard EK et al (1994) Proc Nat Acad Sci 91:3224-7).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, an igif-2 coding sequence may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome will result in a viable virus capable of expressing IGIF-2 in infected host cells. (Logan and Shenk (1984) Proc Natl Acad Sci 81:3655-3659). In addition, transcription enhancers, such as the rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells.

Specific initiation signals may also be required for efficient translation of an

inserted igif-2 coding sequences. These signals include the ATG initiation codon and adjacent sequences. In cases where igif-2, its initiation codon and upstream sequences, are inserted into the appropriate expression vector, no additional translational control signals may be needed. However, in cases where only coding sequence, or a portion thereof, is inserted, exogenous transcriptional control signals, including the ATG initiation codon, must be provided. Furthermore, the initiation codon must be in the correct reading frame to ensure transcription of the entire insert. Exogenous transcriptional elements and initiation codons can be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers appropriate to the cell system (Scharf et al (1994) *Results Probl Cell Differ* 20:125-62; Bittner et al (1987) *Methods Enzymol* 153:516-544).

In addition, a host cell strain may be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation and acylation. Post-translational processing which cleaves a precursor form of the protein may also be important for correct insertion, folding and/or function. Different host cells such as CHO, HeLa, MDCK, 293, WI38, etc have specific cellular machinery and characteristic mechanisms for such post-translational activities and may be chosen to ensure the correct modification and processing of the introduced, foreign protein.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express igif-2 may be transformed using expression vectors which contain viral origins of replication or endogenous expression elements and a selectable marker gene. Following the introduction of the vector, cells may be allowed to grow for 1-2 days in an enriched media, before they are switched to selective media. The selectable marker confers resistance to selection and allows identification of cells which have stably integrated the introduced sequences into their DNA. Resistant clumps of cells can be proliferated using tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell line. These include, but are not limited to, the herpes simplex virus thymidine kinase (Wigler

et al (1977) Cell 11:223) and adenine phosphoribosyltransferase (Lowy et al (1980) Cell 22:817) genes which can be employed in tk⁻ or aprt⁻ cells, respectively. Also, antimetabolite antibiotic or herbicide resistance can be used as the basis of selection; for example, dhfr confers resistance to methotrexate (Wigler et al (1980) Natl Acad Sci 77:3567); npt, which confers resistance to the aminoglycosides, neomycin and G-418 (Colberre-Garapin et al (1981) J Mol Biol 150:1), and als or pat, which confer resistance to chlorsulfuron and phosphinotricin acetyltransferase (Murry, supra). Additional selectable genes have been described, for example, trpB, which allows cells to utilize indole in place of tryptophan, or hisD, which allows cells to utilize histinol in place of histidine (Hartman and Mulligan (1988) Proc Natl Acad Sci 85:8047). Recently, the use of visible markers has gained popularity with such markers as β glucuronidase, anthocyanin, and luciferin being widely used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system (Rhodes CA et al. (1995) Methods Mol Biol 55:121-31).

Identification of Transformants Containing igif-2

Although the presence/absence of marker gene expression suggests that the gene of interest is also present, its presence and expression should be confirmed. For example, if the igif-2 is inserted within a marker gene sequence, recombinant cells containing igif-2 can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with an igif-2 sequence under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of igif-2 as well.

Alternatively, host cells which contain the coding sequence for igif-2 and express IGIF-2 may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridization and protein bioassay or immunoassay techniques which include membrane-based, solution-based, or chip-based technologies for the detection and/or quantification of the nucleic acid or protein.

The presence of the igif-2 polynucleotide sequence can be detected by DNA-DNA or DNA-RNA hybridization or amplification using probes, portions or fragments of igif-

2. Nucleic acid amplification based assays involve the use of oligonucleotides or oligomers based on the igif-2 sequence to detect transformants containing igif-2 DNA or RNA. As used herein "oligonucleotides" or "oligomers" refer to a nucleic acid sequence of at least about 10 nucleotides and as many as about 60 nucleotides, preferably about 15 to 30 nucleotides, and more preferably about 20-25 nucleotides which can be used as a probe or amplimer.

The expression of an IGIF-2 protein product can be assessed biologically in a chemotaxis or Ca^{++} mobilization assay or immunologically in Western blot, enzyme-linked immunoassays (ELISA) and the like.

10 Falk WR et al (1980, J Immunol Methods 33:239) first described the assessment of chemotactic activity using 48-well microchemotaxis chambers. In this assay, the expressed cytokine is placed in media on one side of a polycarbonate filter and a particular population of cells is suspended in the same media on the opposite side of the filter. Sufficient incubation time allows the cells to traverse the filter in response to the cytokine concentration gradient. Filters are recovered from each well, and the cells
15 adhering to the side of the filter facing the cytokine are typed and quantified.

Populations of cells used in such assays may include blood cells obtained from venipuncture or enriched populations of neutrophils, peripheral blood mononuclear cells, monocytes and lymphocytes obtained by density gradient centrifugation and/or negative
20 selection using antibodies specific for surface molecules of the non-desired population. For example, incubating a population of T cells with CD4^{+} and separating out CD4^{+} bound T cells may result in a CD8^{+} enriched T-cell population.

To assay non-chemotactic activity of neutrophils and monocytes, testing may involve measurement of actin polymerization, increase in respiratory burst activity,
25 degranulation of the azurophilic granule or mobilization of Ca^{++} and comparison of the results with standard measurements. The assay for mobilization of Ca^{++} as part of the signal transduction pathway requires preloading neutrophils with a fluorescent probe whose emission characteristics have been altered by Ca^{++} binding. When the cells are exposed to an activating stimulus, Ca^{++} flux is determined by observation of the cells in a
30 fluorometer. The measurement of Ca^{++} mobilization has been described in Grynkievicz G et al (1985) J Biol Chem 260:3440, and McColl S et al (1993) J Immunol 150:4550-4555,

incorporated herein by reference.

Degranulation and respiratory burst responses are similarly measured in monocytes (Zachariae COC et al. (1990) J Exp Med 171: 2177-82). Further measures of monocyte activation are regulation of adhesion molecule expression in lymphocytes (Jiang Y et al (1992) J Immunol 148: 2423-8; Taub D et al (1993) Science 260: 355-358).

A variety of protocols for detecting and measuring the expression of IGIF-2, using either polyclonal or monoclonal antibodies specific for the protein are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA) and fluorescent activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on IGIF-2 is preferred, but a competitive binding assay may be employed. These and other assays are described, among other places, in Hampton R et al (1990, Serological Methods, a Laboratory Manual, APS Press, St Paul MN) and Maddox DE et al (1983, J Exp Med 158:1211).

A wide variety of labels and conjugation techniques are known by those skilled in the art and can be used in various nucleic and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to igif-2 include oligolabeling, nick translation, end-labeling or PCR amplification using a labeled nucleotide. Alternatively, the igif-2 sequence, or any portion of it, may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by addition of an appropriate RNA polymerase such as T7, T3 or SP6 and labeled nucleotides.

A number of companies such as Pharmacia Biotech (Piscataway NJ), Promega (Madison WI), and US Biochemical Corp (Cleveland OH) supply commercial kits and protocols for these procedures. Suitable reporter molecules or labels include those radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents as well as substrates, cofactors, inhibitors, magnetic particles and the like. Patents teaching the use of such labels include US Patents 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149 and 4,366,241. Also, recombinant immunoglobulins may be produced as shown in US Patent No. 4,816,567 incorporated herein by reference.

Purification of IGIF-2

Host cells transformed with an igif-2 nucleotide sequence may be cultured under conditions suitable for the expression and recovery of the encoded protein from cell culture. The protein produced by a recombinant cell may be secreted or may be contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing igif-2 can be designed with signal sequences which direct secretion of IGIF-2 through a particular prokaryotic or eukaryotic cell membrane. Other recombinant constructions may join igif-2 to nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins (Kroll DJ et al (1993) DNA Cell Biol 12:441-53; see also above discussion of vectors containing fusion proteins).

IGIF-2 may also be expressed as a recombinant protein with one or more additional polypeptide domains added to facilitate protein purification. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals (Porath J (1992) Protein Expr Purif 3:263-281), protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp, Seattle WA). The inclusion of a cleavable linker sequences such as Factor XA or enterokinase (Invitrogen, San Diego CA) between the purification domain and IGIF-2 is useful to facilitate purification.

Uses of IGIF-2

Based on its amino acid sequence similarity to IGIF and its expression in lymphocytes, leukocytes and inflamed or cancerous tissues, IGIF-2 would appear to play a role in systemic defense. Therefore, IGIF-2, anti-IGIF-2 antibodies or other small molecules, biological or organic, that modulate the activity of IGIF-2 directly or indirectly can be used therapeutically in the treatment of disease states resulting from abnormal expression of IGIF-2 ; altered leukocyte or lymphocyte activity; or altered immune response.

A therapeutic composition comprising IGIF-2 may have application in the prevention and treatment of individuals subject to diseases or conditions which

compromise the immune system, eg, for example, HIV infection where it would be useful to induce endogenous antiviral molecules, eg, IFN- γ . Given the homology between IGIF (Okamura supra) and IGIF-2, IGIF-2 is expected to stimulate production of IFN γ , and to influence the development of Th1 cells and to favorably modulate the response of Th1 CD4⁺T and CD8⁺T cells in HIV infected individuals. Likewise, the modulation, i.e.,
5 down regulation, of inappropriate Th2 responses or the induction of IFN γ may be useful in treating allergies, particularly in hyperresponsive individuals.

In another embodiment of the present invention, anti-IGIF-2 antibodies capable of neutralizing the activity of IGIF-2 may be used to prevent or treat conditions or disease
10 states such as asthma or septic shock in which tissue destruction results from IGIF-2 expression in combination with the expression of other cytokines. The ability of antibodies or ligands to modulate the effects of IGIF-2 may be measured using the microchemotaxis, Ca⁺⁺ flux or other assays described *infra*.

Procedures well known in the art may be used for the production of antibodies to
15 IGIF-2. Such antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, Fab fragments and fragments produced by a Fab expression library. Neutralizing antibodies, ie, those which inhibit biological activity of IGIF-2, are especially preferred for diagnostics and therapeutics.

For the production of antibodies, various hosts including goats, rabbits, rats, mice,
20 etc may be immunized by injection with IGIF-2 or any portion, fragment or oligopeptide which retains immunogenic properties. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include, but are not limited to, Freund's, mineral gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions,
25 keyhole limpet hemocyanin, and dinitrophenol. BCG (bacilli Calmette-Guerin) and Corynebacterium parvum are potentially useful human adjuvants which may be employed if purified IGIF-2 is administered to immunologically compromised individuals for the purpose of stimulating systemic defense.

Monoclonal antibodies to IGIF-2 may be prepared using any technique which
30 provides for the production of antibody molecules by continuous cell lines in culture. These include but are not limited to the EBV hybridoma technique originally described by

(Kohler G et al (1975) Nature 256:495-497; Kozbor D et al (1985) J Immunol Methods 81:31-42; Cote RJ et al (1983) Proc Natl Acad Sci 80:2026-2030; Cole SP et al (1984) Mol Cell Biol 62:109-120). In addition, techniques developed for the production of "chimeric antibodies", the splicing of mouse antibody genes to human antibody genes to
5 obtain a molecule with appropriate antigen specificity and biological activity can be used (Morrison et al (1984) Proc Natl Acad Sci 81:6851-6855; Neuberger et al (1984) Nature 312:604-608; Takeda et al (1985) Nature 314:452-454). Alternatively, techniques described for the production of single chain antibodies (US Patent No. 4,946,778) can be adapted to produce IGIF-2 specific single chain antibodies.

10 Antibodies may also be produced by inducing in vivo production in the lymphocyte population or by screening recombinant immunoglobulin libraries or panels of highly specific binding reagents as disclosed in Orlandi R et al (1989, Proc Natl Acad Sci 86: 3833-3837), and Winter G and Milstein C (1991; Nature 349:293-299).

15 Antibody fragments which contain specific binding sites for IGIF-2 may also be generated. For example, such fragments include, but are not limited to, the $F(ab')_2$ fragments which can be produced by pepsin digestion of the antibody molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the $F(ab')_2$ fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity (Huse WD
20 et al (1989) Science 256:1275-1281).

IGIF-2-specific antibodies are useful for the diagnosis of conditions and diseases associated with expression of IGIF-2. A variety of protocols for competitive binding or immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve
25 the formation of complexes between IGIF-2 and its specific antibody (or similar IGIF-2-binding molecule) and the measurement of complex formation. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two noninterfering epitopes on a specific IGIF-2 protein is preferred, but a competitive binding assay may also be employed. These assays are described in Maddox DE et al (1983, J Exp Med
30 158:1211).

Diagnostic Assays Using IGIF-2 Specific Antibodies

IGIF-2 antibodies are useful for the diagnosis of conditions, disorders or diseases characterized by abnormal expression of IGIF-2. Diagnostic assays for IGIF-2 include methods utilizing the antibody and a label to detect IGIF-2 in human body fluids, cells, tissues or sections or extracts of such tissues. The polypeptides and antibodies of the present invention may be used with or without modification. Frequently, the polypeptides and antibodies will be labeled by joining them, either covalently or noncovalently, with a reporter molecule. A wide variety of reporter molecules are known, several of which were described above.

A variety of protocols for measuring IGIF-2, using either polyclonal or monoclonal antibodies specific for the respective protein are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA) and fluorescent activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on IGIF-2 is preferred, but a competitive binding assay may be employed. These assays are described, among other places, in Maddox, DE et al (1983, J Exp Med 158:1211).

In order to provide a basis for the diagnosis of disease, normal or standard values for IGIF-2 expression must be established. This is accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with antibody to IGIF-2 under conditions suitable for complex formation which are well known in the art. The amount of standard complex formation may be quantified by comparing it with a dilution series of positive controls where a known amount of antibody is combined with known concentrations of purified IGIF-2. Then, standard values obtained from normal samples may be compared with values obtained from samples from subjects affected by a immunological disorder or disease related to IGIF-2 expression. Deviation between standard and subject values establishes the presence of the disease state.

Drug Screening

IGIF-2, its immunogenic fragments or oligopeptides can be used for screening therapeutic compounds in any of a variety of drug screening techniques. The fragment employed in such a test may be free in solution, affixed to a solid support, borne on a cell

surface, or located intracellularly. The abolition of catalytic activity or the formation of binding complexes, between IGIF-2 and the agent being tested, may be measured.

Another technique for drug screening provides for high throughput screening of compounds having suitable binding affinity to the IGIF-2 polypeptides and is described in detail in Guysen, European Patent Application 84/03564, published on September 13, 1984, incorporated herein by reference. In summary, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The peptide test compounds are reacted with IGIF-2 fragment and washed. Bound IGIF-2 is then detected by methods well known in the art. Purified IGIF-2 can also be coated directly onto plates for use in the aforementioned drug screening techniques. Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support.

This invention also contemplates the use of competitive drug screening assays in which neutralizing antibodies capable of binding IGIF-2 specifically compete with a test compound for binding IGIF-2. In this manner, the antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants with IGIF-2.

Uses of igif-2 Polynucleotide

An igif-2 polynucleotide, or any part thereof, may be used for diagnostic and/or therapeutic purposes. For diagnostic purposes, the igif-2 of this invention may be used to detect and quantitate abnormal gene expression in conditions, disorders or diseases in which igif-2 activity may be implicated. These specifically include, but are not limited to, conditions with similar biochemical or immunological properties such as viral (AIDS, hepatitis), bacterial (septic shock), fungal (histoplasmosis) or helminthic infections; allergies or asthma; mechanical injury through exposure (to asbestos, coal dust, etc) or trauma; arteriosclerosis, atherogenesis or collagen vascular diseases; hereditary diseases such as autoimmune hemolytic anemia, biliary cirrhosis, juvenile diabetes mellitus, lupus erythematosus, multiple sclerosis, myasthenia gravis, or rheumatoid arthritis; leukemia, lymphomas or carcinomas; Crohn's or other inflammatory bowel diseases; or other conditions which involve the abnormal activity of leukocytes or lymphocytes.

Included in the scope of the invention are oligonucleotide sequences, antisense

RNA and DNA molecules and ribozymes. which function to inhibit translation of an igif-2. Such nucleic acid sequences may be used in the treatment of individuals subject to an diseases or conditions associated with inflammation. Another aspect of the subject invention is to provide for hybridization or PCR probes which are capable of detecting polynucleotide sequences, including genomic sequences, encoding IGIF-2 or closely related molecules. The specificity of the probe, ie, whether it is derived from a highly conserved, conserved or non-conserved region or domain, and the stringency of the hybridization or amplification (high, intermediate or low) will determine whether the probe identifies only naturally occurring igif-2, related igif sequences, or other cytokine molecules. Probes for the detection of related nucleic acid sequences are selected from conserved or highly conserved igif-2 sequence. and such probes may be used in a pool of degenerate probes. For the detection of identical nucleic acid sequences. or where maximum specificity is desired, nucleic acid probes are selected from non-conserved nucleotide regions or unique regions of igif-2. As used herein, the term "non-conserved nucleotide region" refers to a nucleotide region that is unique to igif-2 and does not occur in IGIF or other cytokines.

Diagnostic Uses of igif-2 Polynucleotide

An IGIF-2 encoding polynucleotide sequence may be used for the diagnosis of diseases resulting from abnormal expression of igif-2. For example, polynucleotide sequences encoding IGIF-2 may be used in hybridization or PCR assays of tissues from biopsies or autopsies to detect abnormalities in igif-2 expression. The form of such qualitative or quantitative methods may include Southern or northern analysis. dot blot or other membrane-based technologies; PCR technologies; dip stick, pin or chip technologies; and ELISA or other multiple sample format technologies. All of these techniques are well known in the art. and are in fact the basis of many commercially available diagnostic kits.

Such assays may be tailored to evaluate the efficacy of a particular therapeutic treatment regime and may be used in animal studies, in clinical trials, or in monitoring the treatment of an individual patient. In order to provide a basis for the diagnosis of disease, a normal or standard profile for igif-2 expression must be established. This is

accomplished by combining body fluids or cell extracts taken from normal subjects. either animal or human. with igif-2 or a portion thereof. under conditions suitable for hybridization or amplification. Standard hybridization may be quantified by comparing the values obtained for normal subjects with a dilution series of positive controls run in the same experiment where a known amount of purified igif-2 is used. Standard values obtained from normal samples may be compared with values obtained from samples from subjects potentially affected by a disorder or disease related to igif-2 expression. Deviation between standard and subject values establishes the presence of the disease state.

If disease is established, an existing therapeutic agent is administered, and treatment profile or values may be generated. Finally, the assay may be repeated on a regular basis to evaluate whether the values progress toward or return to the normal or standard pattern. Successive treatment profiles may be used to show the efficacy of treatment over a period of several days or several months.

PCR as described in US Patent Nos. 4,683,195; 4,800,195; and 4,965,188 provides additional uses for oligonucleotides based upon the igif-2 sequence. Such oligomers are generally chemically synthesized, but they may be generated enzymatically or produced from a recombinant source. Oligomers generally comprise two nucleotide sequences, one with sense orientation (5'→3') and one with antisense (3'←5') employed under optimized conditions for identification of a specific gene or condition. The same two oligomers, nested sets of oligomers, or even a degenerate pool of oligomers may be employed under less stringent conditions for detection and/or quantitation of closely related DNA or RNA sequences.

Additionally methods to quantitate the expression of a particular molecule include radiolabeling (Melby PC et al 1993 J Immunol Methods 159:235-44) or biotinylating (Duplaa C et al 1993 Anal Biochem 229-36) nucleotides, coamplification of a control nucleic acid, and standard curves onto which the experimental results are interpolated. Quantitation of multiple samples may be speeded up by running the assay in an ELISA format where the oligomer-of-interest is presented in various dilutions and a spectrophotometric or colorimetric response gives rapid quantitation. For example, upregulation of igif-2 may result in an inflammatory response, resulting in swelling and

discomfort. In like manner, underexpression of igif-2 may result in an insufficient immunological response. In either case, a definitive diagnosis may allow health professionals to treat the patient and prevent further worsening of the condition. Similarly, assays known to those of skill in the art can be used to monitor the progress of a patient displaying an igif-2 associated disease state during therapy.

Therapeutic Uses of an igif-2 Polynucleotide

An igif-2 sequence may be useful in the treatment of various abnormal conditions, including diseases or conditions wherein subjects are immunocompromised, eg. HIV infection, where it would be desirable to induce endogenous antiviral molecules. The introduction of the igif-2 sequence into cells can be used to induce IFN- γ thereby stimulating T cell populations. In such instances, the sequence encoding an IGIF-2 is intended to supplement the activity of endogenous cytokines.

Igif-2 antisense constructs may be useful in the treatment of various abnormal conditions characterized by overexpression of igif-2 or other molecules of the immune system. The successful delivery and expression of such sequences to individuals subject to such diseases will reduce or inhibit the transcription of igif-2 mRNA thereby reducing tissue damage resulting from inflammation.

Expression vectors derived from retroviruses, adenovirus, herpes or vaccinia viruses, or from various bacterial plasmids, may be used for delivery of recombinant igif-2, sense or antisense molecules, to the targeted cell population. Methods which are well known to those skilled in the art can be used to construct recombinant vectors containing igif-2. See, for example, the techniques described in Sambrook et al (supra) and Ausubel et al (supra). Alternatively, recombinant igif-2 can be delivered to target cells in liposomes.

The full length cDNA sequence and/or its regulatory elements enable researchers to use igif-2 as a tool in sense (Yousoufian H and HF Lodish 1993 Mol Cell Biol 13:98-104) or antisense (Eguchi et al (1991) Annu Rev Biochem 60:631-652) investigations of gene function. Oligonucleotides, designed from the cDNA or control sequences obtained from the genomic DNA can be used *in vitro* or *in vivo* to inhibit expression. Such technology is now well known in the art, and sense or antisense oligonucleotides or larger

fragments can be designed from various locations along the coding or control regions.

Additionally, igif-2 expression can be modulated by transfecting a cell or tissue with expression vectors which express high levels of an igif-2 fragment. Such constructs can flood cells with untranslatable sense or antisense sequences. Even in the absence of
5 integration into the DNA, such vectors may continue to transcribe RNA molecules until all copies of the vector are disabled by endogenous nucleases. Such transient expression may last for a month or more with a non-replicating vector (Mettler I, personal communication) and even longer if appropriate replication elements are part of the vector system.

10 On the other hand, stable transformation of appropriate germ line cells, or preferably a zygote, with a vector containing the igif-2 fragments may produce a transgenic organism (US Patent No. 4,736,866, 12 April 1988), which produces enough copies of the sense or antisense sequence to significantly compromise or entirely eliminate activity of the endogenous igif-2 gene. Frequently, disruption of such genes can
15 be ascertained by observing behaviors such as reduced inflammatory response or reduced leukocyte proliferation.

As mentioned previously, modifications of gene expression can be obtained by designing antisense sequences to the control regions of the igif-2 gene--the promoters, enhancers, and introns. Oligonucleotides derived from the transcription initiation site, eg,
20 between -10 and +10 regions of the leader sequence, are preferred. Antisense RNA and DNA molecules may also be designed to block translation of mRNA by preventing the transcript from binding to ribosomes. Similarly, inhibition can be achieved using Hogeboom base-pairing methodology, also known as "triple helix" base pairing. Triple helix pairing compromises the ability of the double helix to open sufficiently for the
25 binding of polymerases, transcription factors, or regulatory molecules.

Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. The mechanism of ribozyme action involves sequence specific hybridization of the ribozyme molecule to complementary target RNA, followed by a endonucleolytic cleavage. Within the scope of the invention are engineered hammerhead
30 motif ribozyme molecules that specifically and efficiently catalyze endonucleolytic cleavage of igif-2 RNA sequences.

Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites which include the following sequences, GUA, GUU and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target gene
5 containing the cleavage site may be evaluated for secondary structural features which may render the oligonucleotide sequence inoperable. The suitability of candidate targets may also be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays.

Both antisense RNA and DNA molecules and ribozymes of the invention may be
10 prepared by any method known in the art for the synthesis of RNA molecules. These include techniques for chemically synthesizing oligonucleotides such as solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by *in vitro* or *in vivo* transcription of DNA sequences encoding the antisense RNA molecule. Such DNA sequences may be incorporated into a wide variety of vectors with suitable
15 RNA polymerase promoters such as T7 or SP6. Alternatively, antisense cDNA constructs that synthesize antisense RNA constitutively or inducibly can be introduced into cell lines, cells or tissues.

DNA molecules may be modified to increase intracellular stability and half-life. Possible modifications include, but are not limited to, the addition of flanking sequences
20 of the 5' and/or 3' ends of the molecule or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the backbone of the molecule.

Methods for introducing vectors into cells or tissue include those methods discussed in Section IV of the Examples. In addition, several of these transformation or transfection methods are equally suitable for the *ex vivo* therapy, the introduction of
25 vectors into stem cells taken from the patient and clonally propagated for autologous transplant as described in US Patent Nos. 5,399,493 and 5,437,994, disclosed herein by reference.

Furthermore, the igif-2 polynucleotide sequences disclosed herein may be used in molecular biology techniques that have not yet been developed, provided the new
30 techniques rely on properties of nucleotide sequences that are currently known, including but not limited to such properties as the triplet genetic code and specific base pair

interactions.

Detection and Mapping of Polynucleotide Sequences Related to igif-2

The nucleic acid sequence for igif-2 can also be used to generate hybridization probes as previously described, for mapping the endogenous genomic sequence. The sequence may be mapped to a particular chromosome or to a specific region of the chromosome using well known techniques. These include *in situ* hybridization to chromosomal spreads (Verma et al (1988) Human Chromosomes: A Manual of Basic Techniques, Pergamon Press, New York City), flow-sorted chromosomal preparations, or artificial chromosome constructions such as YACs, bacterial artificial chromosomes (BACs), bacterial P1 constructions or single chromosome cDNA libraries.

In situ hybridization of chromosomal preparations and physical mapping techniques such as linkage analysis using established chromosomal markers are invaluable in extending genetic maps. Examples of genetic maps can be found in Science (1995; 270:410f and 1994; 265:1981f). Often the placement of a gene on the chromosome of another mammalian species may reveal associated markers even if the number or arm of a particular human chromosome is not known. New sequences can be assigned to chromosomal arms, or parts thereof, by physical mapping. This provides valuable information to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once a disease or syndrome, such as ataxia telangiectasia (AT), has been crudely localized by genetic linkage to a particular genomic region, for example, AT to 11q22-23 (Gatti et al (1988) Nature 336:577-580), any sequences mapping to that area may represent associated or regulatory genes for further investigation. The nucleotide sequence of the subject invention may also be used to detect differences in the chromosomal location due to translocation, inversion, etc between normal, carrier or affected individuals.

Pharmaceutical Compositions

The active compositions of the invention, which may comprise all or portions of IGIF-2 or inhibitors or antagonists, including antibodies, alone or in combination with at least one other agent, such as stabilizing compound, may be administered in any sterile,

biocompatible pharmaceutical carrier, including, but not limited to, saline, buffered saline, dextrose, and water.

IGIF-2 can be administered to a patient alone, or in combination with other cytokines, agents, drugs or hormones or in pharmaceutical compositions where it is mixed with excipient(s) or pharmaceutically acceptable carriers. In one embodiment of the present invention, the pharmaceutically acceptable carrier is pharmaceutically inert.

Depending on the condition, disorder or disease being treated, these pharmaceutical compositions may be formulated and administered systemically or locally. Techniques for formulation and administration may be found in the latest edition of "Remington's Pharmaceutical Sciences" (Mack Publishing Co, Easton PA). Suitable routes may, for example, include oral, transvaginal, or transmucosal administration; parenteral delivery, including intramuscular, subcutaneous, intramedullary, intrathecal, intraventricular, intravenous, intraperitoneal, or intranasal administration. The preferred route for IGIF-2 or its inhibitors is intravenous administration.

For injection, the pharmaceutical compositions of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiologically buffered saline. For tissue or cellular administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

The pharmaceutical compositions can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral or nasal ingestion by a patient to be treated.

Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose. Determination of effective amounts is well within the capability of those skilled in the art, especially in light of the disclosure provided below.

In addition to the active ingredients these pharmaceutical compositions may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which

can be used pharmaceutically. The preparations formulated for oral administration may be in the form of tablets, dragees, capsules, or solutions.

The pharmaceutical compositions of the present invention may be manufactured in a manner that is itself known, eg, by means of conventional mixing, dissolving,

5 granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable
10 lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to
15 allow for the preparation of highly concentrated solutions.

Pharmaceutical preparations for oral use can be obtained by combining the active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are carbohydrate or protein fillers such as sugars,
20 including lactose, sucrose, mannitol, or sorbitol; starch from corn, wheat, rice, potato, etc; cellulose such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; and gums including arabic and tragacanth; and proteins such as gelatin and collagen. If desired, disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, alginic acid or a salt thereof such as
25 sodium alginate.

Dragee cores are provided with suitable coatings such as concentrated sugar solutions, which may also contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee
30 coatings for product identification or to characterize the quantity of active compound, ie, dosage.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients mixed with a filler or binders such as lactose or starches, lubricants such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycol with or without stabilizers.

Compositions comprising a compound of the invention formulated in a pharmaceutical acceptable carrier may be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition. For IGIF-2 inhibitors, conditions indicated on the label may include treatment of inflammation.

The pharmaceutical composition may be provided as a salt and can be formed with many acids, including but not limited to hydrochloric, sulfuric, acetic, lactic, tartaric, malic, succinic, etc. Salts tend to be more soluble in aqueous or other protonic solvents that are the corresponding free base forms. In other cases, the preferred preparation may be a lyophilized powder in 1mM-50mM histidine, 0.1%-2% sucrose, 2%-7% mannitol at a pH range of 4.5 to 5.5 that is combined with buffer prior to use.

For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. Then, preferably, dosage can be formulated in animal models to achieve a desirable circulating concentration range that adjusts IGIF-2 levels. Such information can be used to determine useful doses in humans. Examples of animal models useful for studying therapeutic applications of IGIF-2 or its inhibitors include those described in Hutz (1989) Biol Reproduction 40:709-713; Hutz et al. (1990) J Med Primatol 19:553-571 ; Kitzman et al. (1992) Cell Tissue Res 268:191-196; and Quandt et al. (1993) Biol Reprod 48:1088-1094.

A therapeutically effective dose refers to that amount of IGIF-2 or its inhibitor which ameliorates symptoms which may mean stimulation of the immune system and T lymphocytes or the reduction of inflammation and pain. Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, eg, for determining the LD50 (the dose lethal to 50% of

the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index, and it can be expressed as the ratio LD50/ED50. Compounds which exhibit large therapeutic indices are preferred. The data obtained from these cell culture assays and additional animal studies can be used in formulating a range of dosage for human use. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

The exact dosage is chosen by the individual physician in view of the patient to be treated. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Additional factors which may be taken into account include the severity of the disease state; age, weight, and gender of the patient; diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long acting pharmaceutical compositions might be administered every 3 to 4 days, every week, or once every two weeks depending on half-life and clearance rate of the particular formulation.

Normal dosage amounts may vary from 0.1 to 100,000 micrograms, up to a total dose of about 1 g, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature. See US Patent No. 4,657,760; 5,206,344; or 5,225,212. Those skilled in the art will employ different formulations for IGIF-2 than for the inhibitors of IGIF-2. Administration to the lungs may necessitate delivery in a manner different from that to the kidney, or stomach.

Disease states or conditions associated with the immune system which may be treated with nucleic acid or amino acid sequences disclosed herein include, but are not limited to, viral (AIDS, hepatitis), bacterial (septic shock), fungal (histoplasmosis) or helminthic infections; allergies or asthma; mechanical injury through exposure (to asbestos, coal dust, etc) or trauma; arteriosclerosis, atherogenesis or collagen vascular diseases; hereditary diseases such as autoimmune hemolytic anemia, biliary cirrhosis, juvenile diabetes mellitus, lupus erythematosus, multiple sclerosis, myasthenia gravis, or rheumatoid arthritis; leukemia, lymphomas or carcinomas; Crohn's or other inflammatory

bowel diseases: or other conditions which involve the abnormal activity of leukocytes or lymphocytes and which may be specifically diagnosed by the assays previously discussed.

These examples are provided by way of illustration and are not included for the purpose of limiting the invention.

5

INDUSTRIAL APPLICABILITY

I DNA Library Construction

For purposes of discussion, preparation of the kidney cDNA library is described. cDNA libraries from other tissues in which IGIF-2 or its variants are found were prepared by similar methods well known to those of skill in the art.

The kidney cDNA library was constructed from 1.8 micrograms of mRNA made from kidney tissue of a two day old hispanic female (Lot #95-04-0274; International Institute for Advanced Medicine, Exton PA). The tissue was lysed in buffer containing guanidinium isothiocyanate and the lysate was centrifuged over a 5.7 M CsCl cushion using an Beckman SW28 rotor in a Beckman L8-70M Ultracentrifuge (Beckman Instruments) for 18 hours at 25,000 rpm at ambient temperature. The RNA was extracted once with acid phenol pH 4.0, precipitated using 0.3 M sodium acetate and 2.5 volumes of ethanol, resuspended in water and DNase treated for 15 min at 37°C. The poly A⁺ RNA was isolated using the Qiagen Oligotex kit (QIAGEN Inc, Chatsworth CA)

The poly A⁺ RNA was handled according to the recommended protocols in the SuperScript Plasmid System for cDNA Synthesis and Plasmid Cloning (Catalog #18248-013; Gibco/BRL). First strand cDNA synthesis was accomplished using oligo d(T) priming and second strand synthesis was performed using a combination of DNA polymerase I, *E. coli* ligase and RNase H. The cDNA was blunted with T4 polymerase, and a Sal I linker was added to the blunt ended cDNA. The Sal I adapted, double-stranded cDNAs were the digested with Not I and fractionated on a Sepharose CL4B column (Catalog #275105, Pharmacia). Those cDNAs exceeding 400 bp were ligated into pSport I which was subsequently transformed into DH5aTM competent cells (Catalog #18258-012, Gibco/BRL).

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II Isolation and Sequencing of cDNA Clones

Plasmid DNA was released from the cells and purified using the Miniprep Kit (Cat# 77468; Advanced Genetic Technologies Corporation, Gaithersburg MD). This kit consists of a 96 well block with reagents for 960 purifications. The recommended protocol was employed except for the following changes: 1) the 96 wells were each filled with only 1 ml of sterile Terrific Broth (Cat# 22711, LIFE TECHNOLOGIES™, Gaithersburg MD) with carbenicillin at 25 mg/L and glycerol at 0.4%; 2) the bacteria were cultured for 24 hours after the wells were inoculated and then lysed with 60 µl of lysis buffer; 3) a centrifugation step employing the Beckman GS-6R @2900 rpm for 5 min was performed before the contents of the block were added to the primary filter plate; and 4) the optional step of adding isopropanol to TRIS buffer was not routinely performed. After the last step in the protocol, samples were transferred to a Beckman 96-well block for storage.

The cDNAs were sequenced by the method of Sanger F and AR Coulson (1975; J Mol Biol 94:441 f), using a Hamilton Micro Lab 2200 (Hamilton, Reno NV) in combination with four Peltier Thermal Cyclers (PTC200 from MJ Research, Watertown MA) and Applied Biosystems 377 or 373 DNA Sequencing Systems (Perkin Elmer), and the reading frame was determined.

III Homology Searching of cDNA Clones and Their Deduced Proteins

Each cDNA was compared to sequences in GenBank using a search algorithm developed by Applied Biosystems and incorporated into the INHERIT™ 670 Sequence Analysis System. In this algorithm, Pattern Specification Language (TRW Inc, Los Angeles CA) was used to determine regions of homology. The three parameters that determine how the sequence comparisons run were window size, window offset, and error tolerance. Using a combination of these three parameters, the DNA database was searched for sequences containing regions of homology to the query sequence, and the appropriate sequences were scored with an initial value. Subsequently, these homologous regions were examined using dot matrix homology plots to distinguish regions of homology from chance matches. Smith-Waterman alignments were used to display the results of the homology search.

Peptide and protein sequence homologies were ascertained using the INHERIT™

670 Sequence Analysis System in a way similar to that used in DNA sequence homologies. Pattern Specification Language and parameter windows were used to search protein databases for sequences containing regions of homology which were scored with an initial value. Dot-matrix homology plots were examined to distinguish regions of significant homology from chance matches.

BLAST, which stands for Basic Local Alignment Search Tool (Altschul SF (1993) J Mol Evol 36:290-300; Altschul, SF et al (1990) J Mol Biol 215:403-10), was used to search for local sequence alignments. BLAST produces alignments of both nucleotide and amino acid sequences to determine sequence similarity. Because of the local nature of the alignments, BLAST is especially useful in determining exact matches or in identifying homologs. BLAST is useful for matches which do not contain gaps. The fundamental unit of BLAST algorithm output is the High-scoring Segment Pair (HSP).

An HSP consists of two sequence fragments of arbitrary but equal lengths whose alignment is locally maximal and for which the alignment score meets or exceeds a threshold or cutoff score set by the user. The BLAST approach is to look for HSPs between a query sequence and a database sequence, to evaluate the statistical significance of any matches found, and to report only those matches which satisfy the user-selected threshold of significance. The parameter E establishes the statistically significant threshold for reporting database sequence matches. E is interpreted as the upper bound of the expected frequency of chance occurrence of an HSP (or set of HSPs) within the context of the entire database search. Any database sequence whose match satisfies E is reported in the program output.

IV Northern Analysis

Northern analysis (Sambrook et al., supra) was used to detect the presence of an igif-2 transcript among the mRNAs of immunological tissues. Fragments of the igif-2 sequence (SEQ ID NO:1) were randomly primed with radioactive label (see Example and hybridized to a Multiple Tissue Northern blot (MTN; Clontech). Figure 3 shows the image of the resulting igif-2 northern. Expression of igif-2 is clearly shown as a band aligned with standard protein molecular weight marker 1.35 in the lanes containing RNAs from appendix and peripheral blood as well as the lymphoid tissues--spleen, lymph node,

thymus. and bone marrow). The igif-2 transcript was not detected in fetal liver RNA at the stringency used in this experiment.

Analogous electronic northern analysis used BLAST (Altschul, S.F. 1993 and 1990. *supra*) to search for identical or related molecules in the LIFESEQ™ database (Incyte Pharmaceuticals, Inc.). The sensitivity of the computer search was set at a product score of 50. Product score is defined as:

$$\frac{\% \text{ sequence identity} \times \% \text{ maximum BLAST score}}{100}$$

The product score takes into account both the degree of similarity between two sequences and the length of the sequence match. For example, with a product score of 40, the match will be exact within a 1-2% error; and at 70, the match will be exact. Homologous molecules are usually identified by selecting those which show product scores between 15 and 40, although lower scores may identify related molecules.

Figure 4 shows the results of electronic northern analysis reported as a list of libraries in which highly homologous molecules or transcripts encoding IGIF occurred. Abundance and percent abundance are also reported. Abundance directly reflects the number of times a particular transcript is represented in a cDNA library, and percent abundance is abundance divided by the total number of sequences examined in the cDNA library.

V Extension of igif-2 to Recover Regulatory Elements

The nucleic acid sequence of full length igif-2 (SEQ ID NO:1) is used to design oligonucleotide primers for obtaining 5' sequences from genomic libraries. One primer is synthesized to initiate extension in the antisense direction (XLR) and the other is synthesized to extend sequence in the sense direction (XLF). The primers allowed the known igif-2 sequence to be extended "outward" generating amplicons containing new, unknown nucleotide sequence for the control region of interest. The initial primers are designed from the cDNA using Oligo 4.06 (National Biosciences Inc, Plymouth MN), or another appropriate program, to be 22-30 nucleotides in length, to have a GC content of 50% or more, and to anneal to the target sequence at temperatures about 68°-72° C. Any stretch of nucleotides which would result in hairpin structures and primer-primer

dimerizations is avoided.

A human genomic library is used to extend and amplify 5' upstream sequence. If necessary, a second set of primers is designed to further extend the known region. By following the instructions for the XL-PCR kit (Perkin Elmer) and thoroughly mixing the enzyme and reaction mix, high fidelity amplification is obtained. Beginning with 40 pmol of each primer and the recommended concentrations of all other components of the kit. PCR is performed using the Peltier Thermal Cycler (PTC200; MJ Research.

Watertown MA) and the following parameters:

- | | | |
|----|---------|--|
| 10 | Step 1 | 94° C for 1 min (initial denaturation) |
| | Step 2 | 65° C for 1 min |
| | Step 3 | 68° C for 6 min |
| | Step 4 | 94° C for 15 sec |
| | Step 5 | 65° C for 1 min |
| | Step 6 | 68° C for 7 min |
| 15 | Step 7 | Repeat step 4-6 for 15 additional cycles |
| | Step 8 | 94° C for 15 sec |
| | Step 9 | 65° C for 1 min |
| | Step 10 | 68° C for 7:15 min |
| | Step 11 | Repeat step 8-10 for 12 cycles |
| 20 | Step 12 | 72° C for 8 min |
| | Step 13 | 4° C (and holding) |

A 5-10 μ l aliquot of the reaction mixture is analyzed by electrophoresis on a low concentration (about 0.6-0.8%) agarose mini-gel to determine which reactions were successful in extending the sequence. The largest products or bands were selected and cut out of the gel. Further purification involves using a commercial gel extraction method such as QIAQuick™ (QIAGEN Inc). After recovery of the DNA, Klenow enzyme was used to trim single-stranded, nucleotide overhangs creating blunt ends which facilitate religation and cloning.

After ethanol precipitation, the products are redissolved in 13 μ l of ligation buffer, 1 μ l T4-DNA ligase (15 units) and 1 μ l T4 polynucleotide kinase are added, and the mixture is incubated at room temperature for 2-3 hours or overnight at 16° C. Competent *E. coli* cells (in 40 μ l of appropriate media) are transformed with 3 μ l of ligation mixture and cultured in 80 μ l of SOC medium (Sambrook J et al, supra). After incubation for one hour at 37° C, the whole transformation mixture is plated on Luria Bertani (LB)-agar (Sambrook J et al. supra) containing 2x Carb. The following day, several colonies are

randomly picked from each plate and cultured in 150 μ l of liquid LB/2xCarb medium placed in an individual well of an appropriate, commercially-available, sterile 96-well microtiter plate. The following day, 5 μ l of each overnight culture is transferred into a non-sterile 96-well plate and after dilution 1:10 with water, 5 μ l of each sample is transferred into a PCR array.

For PCR amplification, 18 μ l of concentrated PCR reaction mix (3.3x) containing 4 units of rTth DNA polymerase, a vector primer and one or both of the gene specific primers used for the extension reaction are added to each well. Amplification is performed using the following conditions:

- | | | |
|----|--------|--|
| 10 | Step 1 | 94° C for 60 sec |
| | Step 2 | 94° C for 20 sec |
| | Step 3 | 55° C for 30 sec |
| | Step 4 | 72° C for 90 sec |
| | Step 5 | Repeat steps 2-4 for an additional 29 cycles |
| 15 | Step 6 | 72° C for 180 sec |
| | Step 7 | 4° C (and holding) |

Aliquots of the PCR reactions are run on agarose gels together with molecular weight markers. The sizes of the PCR products are compared to the original partial cDNAs, and appropriate clones are selected, ligated into plasmid and sequenced.

20

VI Labeling of Hybridization Probes

Hybridization probes derived from SEQ ID NO:1 are employed to screen cDNAs, mRNAs or genomic DNAs. Although the labeling of oligonucleotides, consisting of about 20 base-pairs, is specifically described, essentially the same procedure is used with larger cDNA fragments. Oligonucleotides are labeled by combining 50 pmol of each oligomer and 250 mCi of [γ -³²P] adenosine triphosphate (Amersham, Chicago IL) and T4 polynucleotide kinase (DuPont NEN®, Boston MA). The labeled oligonucleotides are purified with Sephadex G-25 super fine resin column (Pharmacia). A portion containing 10⁷ counts per minute of each is used in a typical membrane based hybridization analysis of human genomic DNA digested with one of the following endonucleases (Ase I, Bgl II, EcoR I, Pst I, Xba 1, or Pvu II; DuPont NEN®).

30

The DNA from each digest is fractionated on a 0.7 percent agarose gel and transferred to nylon membranes (Nytran Plus, Schleicher & Schuell, Durham NH).

Hybridization is carried out for 16 hours at 40°C. To remove nonspecific signals, blots are sequentially washed at room temperature under increasingly stringent conditions up to 0.1 x saline sodium citrate and 0.5% sodium dodecyl sulfate. After XOMAT ART™ film (Kodak, Rochester NY) is exposed to the blots in a Phosphoimager cassette (Molecular Dynamics, Sunnyvale CA) for several hours, hybridization patterns are compared visually.

VII Antisense Molecules

The complement of the igif-2 sequence, or any part thereof, is used to inhibit *in vivo* or *in vitro* expression of endogenous igif-2. Although use of antisense oligonucleotides, consisting of about 20 base-pairs, is specifically described, essentially the same procedure is used with larger cDNA fragments. An oligonucleotide complementary to the coding sequence of igif-2 is used to inhibit expression of endogenous igif-2. Using Oligo 4.06, the complementary oligonucleotide is designed from the conserved 5' sequence and used to inhibit transcription or from 3' sequence and used to prevent the ribosome from translating the mRNA.

VIII Cloning and Expression of IGIF-2

Nucleotides for a Kozak sequence were engineered upstream of the initiating ATG, and nucleotides encoding a six residue histidine tag were added to the 3' end of SEQ ID NO:2. The optimized nucleotide sequence encoding IGIF-2 was cloned into the pCEP vector (Invitrogen). Lipofectamine (Gibco BRL) was used to transform pCEP into 293 cells which had been transformed previously with EBNA (Invitrogen). Stable transformants were selected using 300 ug/ml hygromycin. The native signal sequence of IGIF provides for secretion of the protein into the growth media which is used in the test for IGIF-2 activity.

IX IGIF-2 Activity

Cytokine chemotactic activity is usually measured in 48-well microchemotaxis chambers. In each well, two compartments are separated by a filter that allows the passage of cells from one compartment into the other in response to a chemical gradient.

Cell culture medium into which IGIF-2 has been secreted is placed on one side of a polycarbonate filter, and peripheral blood cells are suspended in the same media opposite side of the filter. Sufficient incubation time is allowed for the cells to traverse the filter in response to diffusion and resulting concentration gradient of IGIF-2. Filters are recovered from each well, and specific cell types, eg, monocytes, adhering to the side of the filter facing the cytokine are identified and counted.

Specificity of the chemoattraction is determined by performing the assay on fractionated populations of cells such as enriched populations of monocytes or lymphocytes obtained by density gradient centrifugation. Specific T cell populations are further purified using CD8+ and CD4+ specific antibodies for negative selection.

X Production of IGIF-2 Specific Antibodies

Although IGIF-2 purified using PAGE electrophoresis (Sambrook et al, supra) may be used to immunize rabbits using standard protocols, a monoclonal approach is more commonly employed. The amino acid sequence translated from igif-2 is analyzed using DNASTAR software (DNASTAR Inc) to determine regions of high immunogenicity and a corresponding oligopeptide is synthesized and used to raise antibodies by means known to those of skill in the art. Analysis to select appropriate epitopes, such as those near the C-terminus or in adjacent hydrophilic regions is described by Ausubel FM et al (supra).

Typically, the oligopeptides are 15 residues in length, synthesized using an Applied Biosystems Peptide Synthesizer Model 431A using fmoc-chemistry, and coupled to keyhole limpet hemocyanin (KLH, Sigma) by reaction with M-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS; Ausubel FM et al, supra). Rabbits are immunized with the oligopeptide-KLH complex in complete Freund's adjuvant. The resulting antisera are tested for antipeptide activity, for example, by binding the peptide to plastic, blocking with 1% BSA, reacting with rabbit antisera, washing, and reacting with radioiodinated, goat anti-rabbit IgG.

XI Purification of IGIF-2 Using Specific Antibodies

Immunoaffinity chromatography is used to purify endogenous or recombinant

IGIF-2 using antibodies specific for IGIF-2. An immunoaffinity column is constructed by covalently coupling IGIF-2 antibody to an activated chromatographic resin such as CnBr-activated Sepharose (Pharmacia Biotech). After the coupling, the resin is blocked and washed according to the manufacturer's instructions.

Media containing IGIF-2 is passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of IGIF-2 (eg, high ionic strength buffers in the presence of detergent). The column is eluted under conditions that disrupt antibody/IGIF-2 binding (eg, a buffer of pH 2-3 or a high concentration of a chaotrope such as urea or thiocyanate ion), and IGIF-2 is collected.

XII Identification of Molecules Which Interact with IGIF-2

IGIF-2, or biologically active fragments thereof, are labeled with ¹²⁵I Bolton-Hunter reagent (Bolton, AE and Hunter, WM (1973) Biochem J 133: 529). Candidate small molecules previously arrayed in the wells of a 96 well plate are incubated with the labeled IGIF-2, washed and any wells with labeled IGIF-2 complex are assayed. Data obtained using different concentrations of IGIF-2 are used to calculate values for the number, affinity, and association of IGIF-2 with the candidate molecules.

All publications and patents mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described method and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: INCYTE PHARMACEUTICALS, INC.

(ii) TITLE OF THE INVENTION: NUCLEIC ACIDS ENCODING INTERFERON GAMMA
INDUCING FACTOR-2

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:

- (A) ADDRESSEE: Incyte Pharmaceuticals, Inc.
- (B) STREET: 3174 Porter Drive
- (C) CITY: Palo Alto
- (D) STATE: CA
- (E) COUNTRY: USA
- (F) ZIP: 94304

(v) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Diskette
- (B) COMPUTER: IBM Compatible
- (C) OPERATING SYSTEM: DOS
- (D) SOFTWARE: FastSEQ for Windows Version 2.0

(vi) CURRENT APPLICATION DATA:

- (A) PCT APPLICATION NUMBER: To Be Assigned
- (B) FILING DATE: Herewith
- (C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

- (A) APPLICATION SERIAL NUMBER: US 08/580,667
- (B) FILING DATE: 29-DEC-1995

(viii) ATTORNEY/AGENT INFORMATION:

- (A) NAME: Billings, Lucy J.
- (B) REGISTRATION NUMBER: 36,749
- (C) REFERENCE/DOCKET NUMBER: PF-0051 PCT

(ix) TELECOMMUNICATION INFORMATION:

- (A) TELEPHONE: 415-855-0555
- (B) TELEFAX: 415-845-4166
- (C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1101 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:

- (A) LIBRARY: Kidney
- (B) CLONE: 631796

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```

GTCGACCCAC GCGTCCGCTG GCGACTGCCT GGACAGTCAG CAAGGAATTG TCTCCAGTG      60
CATTTTGGCC TCCTGGCTGC CAACGCTGGC TGCTAAAGTG GCTGCCACCT GCTGCAGTCT      120
ACACAGCTTC GGAAGAGGA AAGGAACCTC AGACCTTCCA GATCGCTTCC TCTCGCAACA      180
AACTATTGCT CGCAGGAATA AAGATGGCTG CTGAACCAGT AGAAGACAAT TGCATCAACT      240
TTGTGGCAAT GAAATTTATT GACAATACGC TTTACTTTAT AGCTGAAGAT GATGAAAACC      300
TGGAATCCGA TTACTTTGGC AAGCTTGAAT CTAAATTATC AGTCATAAGA AATTTGAATG      360
ACCAAGTTCT CTTCAATTGAC CAAGGAAATC GGCCTCTATT TGAAGATATG ACTGATTCTG      420
ACTGTAGAGA TAATGCACCC CGGACCATAT TTATTATAAG TATGTATAAA GATAGCCAGC      480
CTAGAGGTAT GGCTGTAAT ATCTCTGTGA AGTGTGAGAA AATTTCAACT CTCTCCTGTG      540
AGAACAAAAT TATTTCTTTT AAGGAAATGA ATCCTCCTGA TAACATCAAG GATACAAAAA      600
GTGACATCAT ATTCTTTTCA AGAAGTGTCC CAGGACATGA TAATAAGATG CAATTTGAAT      660
CTTCATCATA CGAAGGATAC TTTCTAGCTT GTGAAAAAGA GAGAGACCTT TTTAAACTCA      720
TTTTGAAAAA AGAGGATGAA TTGGGGGATA GATCTATAAT GTTCACTGTT CAAAACGAAG      780
ACTAGCTATT AAAATTTTCA GCCGGGCGCA GTGGCTCACG CCTGTAATCC CAGCCCTTTG      840
GGAGGCTGAG GCGGGCAGAT CACCAGAGGT CAGGTGTTCA AGACCAGCCT GACCAACATG      900
GTGAAACCTC ATCTCTACTA GAAATACAAA AAATTAGCTG AGTGTAGTGA CCCATGCCCT      960
CAATCCCAGC TACTCAAGAG GCTGAGGCAG GAGAATCACT TGCCTCCGG AGGTGGAGGT     1020
TGTGGTGAGC CGAGATTGCA CCATTGCGCT CTAGCCTGGG CAACAACAGC CAAACTCCAT     1080
CTCCAAAAAA AAAAAAAAAA A

```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 193 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:

- (A) LIBRARY: Kidney
- (B) CLONE: 631796

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```

Met Ala Ala Glu Pro Val Glu Asp Asn Cys Ile Asn Phe Val Ala Met
 1          5          10          15
Lys Phe Ile Asp Asn Thr Leu Tyr Phe Ile Ala Glu Asp Asp Glu Asn
 20          25          30
Leu Glu Ser Asp Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile
 35          40          45
Arg Asn Leu Asn Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro
 50          55          60
Leu Phe Glu Asp Met Thr Asp Ser Asp Cys Arg Asp Asn Ala Pro Arg
 65          70          75          80
Thr Ile Phe Ile Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met
 85          90          95
Ala Val Thr Ile Ser Val Lys Cys Glu Lys Ile Ser Thr Leu Ser Cys
100          105          110
Glu Asn Lys Ile Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile
115          120          125
Lys Asp Thr Lys Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly
130          135          140
His Asp Asn Lys Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe
145          150          155          160
Leu Ala Cys Glu Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys
165          170          175
Glu Asp Glu Leu Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu
180          185          190
Asp

```


(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 12 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:

- (A) LIBRARY: adenoid
- (B) CLONE: 159939

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Gly Lys Val Glu Met Asn Leu Phe Phe Phe Ala Asn
 1 5 10

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 192 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:

- (A) LIBRARY: GenBank
- (B) CLONE: 1064823

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Ala Ala Met Ser Glu Asp Ser Cys Val Asn Phe Lys Glu Met Met
 1 5 10 15
 Phe Ile Asp Asn Thr Leu Tyr Phe Ile Pro Glu Glu Asn Gly Asp Leu
 20 25 30
 Glu Ser Asp Asn Phe Gly Arg Leu His Cys Thr Thr Ala Val Ile Arg
 35 40 45
 Asn Ile Asn Asp Gln Val Leu Phe Val Asp Lys Arg Gln Pro Val Phe
 50 55 60
 Glu Asp Met Thr Asp Ile Asp Gln Ser Ala Ser Glu Pro Gln Thr Arg
 65 70 75 80
 Leu Ile Ile Tyr Met Tyr Lys Asp Ser Glu Val Arg Gly Leu Ala Val
 85 90 95
 Thr Leu Ser Val Lys Asp Ser Lys Met Ser Thr Leu Ser Cys Lys Asn
 100 105 110
 Lys Ile Ile Ser Phe Glu Glu Met Asp Pro Pro Glu Asn Ile Asp Asp
 115 120 125
 Ile Gln Ser Asp Leu Ile Phe Phe Gln Lys Arg Val Pro Gly His Asn
 130 135 140
 Lys Met Glu Phe Glu Ser Ser Leu Tyr Glu Gly His Phe Leu Ala Cys
 145 150 155 160
 Gln Lys Glu Asp Asp Ala Phe Lys Leu Ile Leu Lys Lys Lys Asp Glu
 165 170 175
 Asn Gly Asp Lys Ser Val Met Phe Thr Leu Thr Asn Leu His Gln Ser
 180 185 190

CLAIMS

1. A purified polynucleotide comprising a nucleic acid sequence encoding interferon gamma inducing factor-2 (IGIF-2) of SEQ ID NO:2.
- 5 2. The polynucleotide of claim 1 wherein the polynucleotide sequence consists of SEQ ID NO:1.
3. The purified polynucleotide of claim 1 wherein said polypeptide has isoleucine at residue 140.
4. The polynucleotide of claim 3 wherein the polynucleotide sequence of SEQ ID
10 NO:1 has thymine at position 622.
5. A purified polynucleotide comprising a nucleic acid sequence encoding the polypeptide having amino acid residues 1-30 of SEQ ID NO:2 followed by the amino acid residues of SEQ ID NO:3.
6. An antisense molecule comprising the complement of the polynucleotide of claim
15 2 or a portion thereof.
7. An expression vector comprising the polynucleotide of claim 1.
8. A host cell transformed with the expression vector of claim 7.
9. A purified polypeptide comprising the amino acid sequence of SEQ ID NO:2.
10. The purified polypeptide of claim 9 having isoleucine at amino acid residue 140.
- 20 11. A purified polypeptide comprising amino acid residues 1-30 of claim 9 followed by the amino acid residues of SEQ ID NO:3.
12. A pharmaceutical composition comprising an effective amount of the antisense molecule of claim 6 and a pharmaceutically acceptable excipient.
13. A method of treating a subject having a condition associated with altered igif-2
25 expression comprising administering an effective amount of the pharmaceutical composition of claim 12 to the subject.
14. A diagnostic composition comprising an oligonucleotide probe of the polynucleotide of claim 2.
15. A diagnostic test for the detection of nucleotide sequences encoding IGIF-2 in a
30 biological sample, comprising the steps of:
 - a) combining the biological sample with a first nucleotide sequence which

comprises the nucleotide sequence of claim 2, or a fragment thereof, under conditions suitable for the formation of a nucleic acid hybridization complex,

5 b) detecting said hybridization complex, wherein the presence of said complex correlates with the presence of a second nucleotide sequence encoding IGIF-2 in said biological sample, and

c) comparing the amount of the second nucleotide sequence in said sample with a standard thereby determining whether the amount of said second nucleotide sequence varies from said standard, wherein the presence of an abnormal level of said second nucleotide sequence correlates positively with a condition associated with inflammation or aberrant expression of IGIF-2.

10 16. A method of screening a plurality of compounds for specific binding affinity with the IGIF-2 or a portion thereof comprising the steps of:

a) providing a plurality of compounds;

b) combining the polypeptide of claim 9 with each of a plurality of compounds for 15 a time sufficient to allow binding under suitable conditions; and

c) detecting binding of IGIF-2 to each of the plurality of compounds, thereby identifying the compounds which specifically bind IGIF-2.

17. A method for producing IGIF-2, said method comprising the steps of:

a) culturing the host cell of claim 8 under conditions suitable for the expression of 20 said polypeptide, and

b) recovering said polypeptide from the host cell culture.

18. An antagonist specifically binding the polypeptide of claim 9 or a portion thereof.

19. A pharmaceutical composition comprising the antagonist of claim 18 and a pharmaceutically acceptable excipient.

25 20. A method of treating a subject with a condition associated with altered IGIF-2 expression comprising administering an effective amount of the pharmaceutical composition of claim 19 to the subject.

1 / 5

9 18 27 36 45 54
 5' NGT CGA CCC ACG CGT CCG CTG GCG ACT GCC TGG ACA GTC AGC AAG GAA TTG TCT
 63 72 81 90 99 108
 CCC AGT GCA TTT TGC CCT CCT GGC TGC CAA CGC TGG CTG CTA AAG TGG CTG CCA
 117 126 135 144 153 162
 CCT GCT GCA GTC TAC ACA GCT TCG GGA AGA GGA AAG GAA CCT CAG ACC TTC CAG
 171 180 189 198 207 216
 ATC GCT TCC TCT CGC AAC AAA CTA TTT GTC GCA GGA ATA AAG ATG GCT GCT GAA
 M A A E
 225 234 243 252 261 270
 CCA GTA GAA GAC AAT TGC ATC AAC TTT GTG GCA ATG AAA TTT ATT GAC AAT ACG
 P V E D N C I N F V A M K F I D N T
 279 288 297 306 315 324
 CTT TAC TTT ATA GCT GAA GAT GAT GAA AAC CTG GAA TCC GAT TTT GGC AAG
 L Y F I A E D D E N L E S D Y F G K
 333 342 351 360 369 378
 CTT GAA TCT AAA TTA TCA GTC ATA AGA AAT TTG AAT GAC CAA GTT CTC TTC ATT
 L E S K L S V I R N L N D Q V L F I
 387 396 405 414 423 432
 GAC CAA GGA AAT CGG CCT CTA TTT GAA GAT ATG ACT GAT TCT GAC TGT AGA GAT
 D Q G N R P L F E D M T D S D C R D
 441 450 459 468 477 486
 AAT GCA CCC CGG ACC ATA TTT ATT ATA AGT ATG TAT AAA GAT AGC CAG CCT AGA
 N A P R T I F I I S M Y K D S Q P R
 495 504 513 522 531 540
 GGT ATG GCT GTA ACT ATC TCT GTG AAG TGT GAG AAA ATT TCA ACT CTC TCC TGT
 G M A V T I S V K C E K I S T L S C
 549 558 567 576 585 594
 GAG AAC AAA ATT ATT TCC TTT AAG GAA ATG AAT CCT CCT GAT AAC ATC AAG GAT
 E N K I I S F K E M N P P D N I K D
 603 612 621 630 639 648
 ACA AAA AGT GAC ATC ATA TTC TTT CAG AGA AGT GTC CCA GGA CAT GAT AAT AAG
 T K S D I I F F Q R S V P G H D N K
 657 666 675 684 693 702
 ATG CAA TTT GAA TCT TCA TCA TAC GAA GGA TAC TTT CTA GCT TGT GAA AAA GAG
 M Q F E S S S Y E G Y F L A C E K E

FIGURE 1A

2 / 5

711	720	729	738	747	756
AGA GAC CTT TTT AAA CTC ATT TTG AAA AAA GAG GAT GAA TTG GGG GAT AGA TCT					
R D L F K L I L K K E D E L G D R S					
765	774	783	792	801	810
ATA ATG TTC ACT GTT CAA AAC GAA GAC TAG CTA TTA AAA TTT CAT GCC GGG CGC					
I M F T V Q N E D					
819	828	837	846	855	864
AGT GGC TCA CGC CTG TAA TCC CAG CCC TTT GGG AGG CTG AGG CGG GCA GAT CAC					
873	882	891	900	909	918
CAG AGG TCA GGT GTT CAA GAC CAG CCT GAC CAA CAT GGT GAA ACC TCA TCT CTA					
927	936	945	954	963	972
CTA GAA ATA CAA AAA ATT AGC TGA GTG TAG TGA CCC ATG CCC TCA ATC CCA GCT					
981	990	999	1008	1017	1026
ACT CAA GAG GCT GAG GCA GGA GAA TCA CTT GCA CTC CGG AGG TGG AGG TTG TGG					
1035	1044	1053	1062	1071	1080
TGA GCC GAG ATT GCA CCA TTG CGC TCT AGC CTG GGC AAC AAC AGC CAA ACT CCA					
1089	1098				
TCT CCA AAA AAA AAA AAA AAA A 3'					

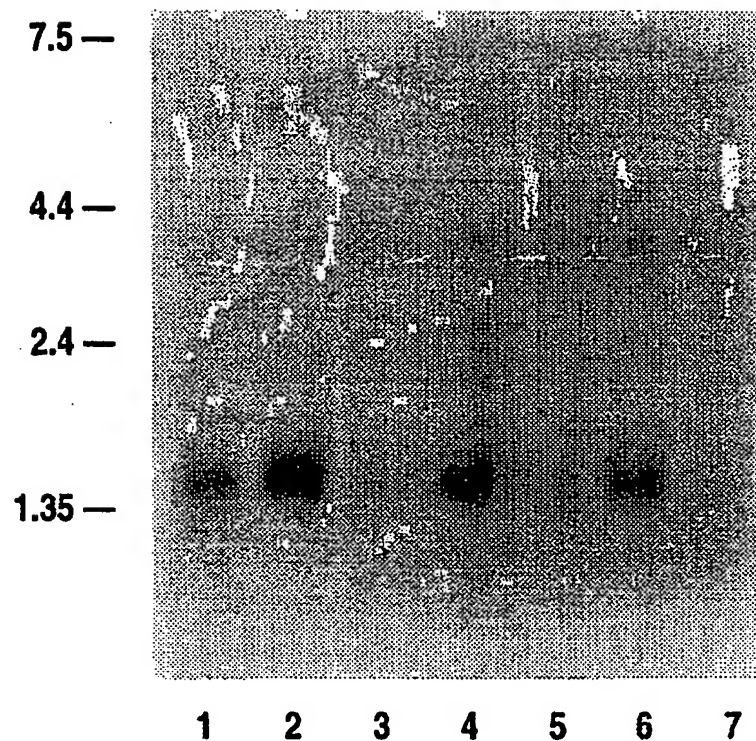
FIGURE 1B

3 / 5

1	MAAEPVEDNCIN	10	20	30	40	631796
1	MAAAMS-EDSCVNEKE					GI 1064823
41	LESKLSVIRNLNDQVLFI	50	60	70	80	631796
40	LHCTTAVIRNINDQVLE					GI 1064823
81	TIFFIISMYKDSQPRGMAVTISVKCEKISTLSCE	90	100	110	120	631796
79	TRLIIVMYKDSSEVRGLAVTLSVNDKMS					GI 1064823
121	EMNPPDNIKD	130	140	150	160	631796
119	EMDPPENNDDIQSDLI					GI 1064823
161	LACEKERD	170	180	190		631796
158	LACQKE					GI 1064823

FIGURE 2

4 / 5

**FIG. 3**

5 / 5

Library	Lib Description	Abun	Pct Abun

SKINBIT01	skin, leg, erythema nodosum	1	0.0256
TLYMNOT02	lymphocytes (non-adher PBMNC), M/F	1	0.0254
LIVRNOT01	liver, 49 M	1	0.0198
ADENINB01	adenoid, inflamed, 3y	1	0.0190
SYNORAT04	synovium, wrist, rheumatoid, 62 F	1	0.0174
BEPINOT01	bronchial epithelium, primary cell line, 54 M	1	0.0144
KIDNNOT05	kidney, neonatal F	1	0.0106
LUNGAST01	lung, asthma, 17 M	1	0.0094
BRAITUT02	brain tumor, metastasis, 58 M	1	0.0075
BRAITUT03	brain tumor, astrocytoma, 17 F	1	0.0074

FIGURE 4

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/20432

A. CLASSIFICATION OF SUBJECT MATTER					
IPC 6	C12N15/19	C12N15/11	C12N15/85	C07K14/52	C12N5/10
	C12Q1/68	C12P21/02	A61K38/19	A61K48/00	G01N33/53
According to International Patent Classification (IPC) or to both national classification and IPC					
B. FIELDS SEARCHED					
Minimum documentation searched (classification system followed by classification symbols)					
IPC 6 C12N C07K C12Q C12P A61K G01N					
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched					
Electronic data base consulted during the international search (name of data base and, where practical, search terms used)					
C. DOCUMENTS CONSIDERED TO BE RELEVANT					
Category *	Citation of document, with indication, where appropriate, of the relevant passages				Relevant to claim No.
X	NATURE, vol. 378, 2 November 1995, pages 88-91, XP002030033 OKAMURA H. ET AL.: "Cloning of a new cytokine that induces IFN- γ production by T cells" cited in the application				6
A	see the whole document				1-5,7-20
A	INFECTION AND IMMUNITY, vol. 63, no. 10, October 1995, pages 3966-3972, XP002030034 OKAMURA H. ET AL.: "A novel costimulatory factor for gamma interferon induction found in the livers of mice causes endotoxic shock" see the whole document				1-20
-/--					
<input checked="" type="checkbox"/> Further documents are listed in the continuation of box C. <input checked="" type="checkbox"/> Patent family members are listed in annex.					
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "&" document member of the same patent family					
Date of the actual completion of the international search			Date of mailing of the international search report		
23 April 1997			02. 05. 97		
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016			Authorized officer Kania, T		

INTERNATIONAL SEARCH REPORT

International Application No

PC1/US 96/20432

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	THE JOURNAL OF IMMUNOLOGY, vol. 156, no. 11, 1 June 1996, pages 4274-4279, XP002030035 USHIO,S. ET AL.: "Cloning of the cDNA for human IFN-γ-inducing factor, expression in Escherichia coli, and studies on the biologic activities of the protein" see the whole document ---	1,2,7-9
P,X	EP 0 712 931 A (HAYASHIBARA BIOCHEM LAB) 22 May 1996 see the whole document ---	1,2,7-9, 14,15, 17-20
E	EP 0 767 178 A (HAYASHIBARA BIOCHEM LAB) 9 April 1997	1,2,7-9
T	* the whole document, esp. page 2, lines 12- 26 * -----	3-5,10, 11

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 96/20432

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 13,20
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although these claims (as far as in vivo methods are concerned) are directed to a method of treatment of (diagnostic method practised on) the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No

PC1/US 96/20432

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